SYNTHESIS OF GLYCOPEPTIDES WITH SUPERIOR PHARMACOKINETIC PROPERTIES

RELATED APPLICATIONS

The present application is related to, and claims priority from, United States Provisional Patent Application No. 60/516,838, filed November 4, 2003, United States Provisional Patent Application No. 60/557,631, filed March 30, 2004, and United States Provisional Patent Application No. 60/598,215, filed August 2, 2004, the entire disclosures of which are herein incorporated by reference.

FIELD OF INVENTION

The present invention relates generally to novel glycopeptides having improved pharmacokinetic properties, methods of producing the same, and methods of using the same. The present invention also relates to novel glycopeptides in which a particular sugar motif can serve as a stable surrogate for a specific amino acid residue.

carbohydrate and an amino, hydroxyl or carboxyl group on the peptide, thereby significantly increasing the half-life of said peptide in a biological system.

To the best of our knowledge, there is no precedent in literature or prior-art, which teaches that a particular sugar motif can serve as a stable surrogate of a specific amino acid residue. The structures of the carbohydrate moieties (sugar motifs) vary from natural neutral-, amino-, and acidic-sugars to those with five- or six-membered sugar frameworks with a wide variety of functional groups that are combinatorially generated.

Specifically, the introduced sugar motif can replace the serine residue if the sugar motif has a hydroxyl group, or replace basic amino acid residues, including lysine, arginine, or histidine, if the sugar motif contains a basic functionality such as an amino group.

Definitions

The compounds of the invention comprise asymmetrically substituted carbon atoms. Such asymmetrically substituted carbon atoms can result in the compounds of the invention comprising mixtures of stereoisomers at a particular asymmetrically substituted carbon atom or a single stereoisomer. As a result, racemic mixtures, mixtures of diastereomers, as well as single diastereomers of the compounds of the invention are included in the present invention. The terms "*S*" and "*R*" configuration, as used herein, are as defined by the IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry, Pure Appl. Chem. (1976) 45, 13-30.

The compositions containing the compound(s) of the invention can be administered for prophylactic and/or therapeutic treatments. An amount adequate to accomplish this is defined as "therapeutically effective amount or dose." Amounts effective for this use will depend on the severity and course of the disease or condition, previous therapy, the patient's health status and response to the drugs, and the judgment of the treating physician. In prophylactic applications, compositions containing the compounds of the invention are administered to a patient susceptible to or otherwise at risk of a particular disease or condition. Such an amount is defined to be a "prophylactically effective amount or dose." In this use, the precise amounts again depend on the patient's state of health, weight, and the like.

Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of

combination thereof. If part of a linker and that linker comprises one or more rings as part of the core backbone, for purposes of calculating chain length, the "chain" only includes those carbon atoms that compose the bottom or top of a given ring and not both, and where the top and bottom of the ring(s) are not equivalent in length, the shorter distance shall be used in determining chain length. If the chain contains heteroatoms as part of the backbone, those atoms are not calculated as part of the carbon chain length.

The term "physiologically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

The term "excipient" refers to an inert substance added to a pharmacological composition to further facilitate administration of a compound. Examples of excipients include but are not limited to, calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

The term "alkyl", alone or in combination, refers to an optionally substituted straight-chain, optionally substituted branched-chain, or optionally substituted cyclic alkyl radical having from 1 to about 30 carbons (e.g., C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, $C2_1$, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}), preferably 1 to 12 carbons (e.g., C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}). Examples of alkyl radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, tert-amyl, pentyl, hexyl, heptyl, octyl and the like.

The term "cycloalkyl" embraces cyclic configurations, is subsumed within the definition of alkyl and specifically refers to a monocyclic, bicyclic, tricyclic, and higher multicyclic alkyl radicals wherein each cyclic moiety has from 3 to about 8 carbon atoms (e.g., C_3, C_4, C_5, C_6, C_7, C_8). Examples of cycloalkyl radicals include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

A "lower alkyl" is a shorter alkyl, *e.g.*, one containing from 1 to about 6 carbon atoms (e.g., C_1, C_2, C_3, C_4, C_5, C_6).

The term "alkenyl," alone or in combination, refers to an optionally substituted straight-chain, optionally substituted branched-chain, or optionally substituted cyclic alkenyl hydrocarbon radical having one or more carbon-carbon double-bonds and having from 2 to about 30 carbon atoms (e.g., C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11},

The term "aryl," alone or in combination, refers to an optionally substituted aromatic ring system. The term aryl includes monocyclic aromatic rings, polyaromatic rings and polycyclic aromatic ring systems containing from six to about twenty carbon atoms. The term aryl also includes monocyclic aromatic rings, polyaromatic rings and polycyclic ring systems containing from 6 to about 12 carbon atoms (e.g., C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}), as well as those containing from 6 to about 10 carbon atoms (e.g., C_6, C_7, C_8, C_9, C_{10}). The polyaromatic and polycyclic aromatic rings systems may contain from two to four rings. Examples of aryl groups include, without limitation, phenyl, biphenyl, naphthyl and anthryl ring systems.

The term "heteroaryl" refers to optionally substituted aromatic ring systems containing from about five to about 20 skeletal ring atoms and having one or more heteroatoms such as, for example, oxygen, nitrogen, sulfur, and phosphorus. The term heteroaryl also includes optionally substituted aromatic ring systems having from 5 to about 12 skeletal ring atoms, as well as those having from 5 to about 10 skeletal ring atoms. The term heteroaryl may include five- or six-membered heterocyclic rings, polycyclic heteroaromatic ring systems and polyheteroaromatic ring systems where the ring system has two, three or four rings. The terms heterocyclic, polycyclic heteroaromatic and polyheteroaromatic include ring systems containing optionally substituted heteroaromatic rings having more than one heteroatom as described above (*e.g.*, a six membered ring with two nitrogens), including polyheterocyclic ring systems of from two to four rings. The term heteroaryl includes ring systems such as, for example, furanyl, benzofuranyl, chromenyl, pyridyl, pyrrolyl, indolyl, quinolinyl, *N*-alkyl pyrrolyl, pyridyl-*N*-oxide, pyrimidoyl, pyrazinyl, imidazolyl, pyrazolyl, oxazolyl, benzothiophenyl, purinyl, indolizinyl, thienyl and the like.

The term "heteroarylalkyl" refers to a C_1-C_4 alkyl group (e.g., C_1, C_2, C_3, C_4) containing a heteroaryl group, each of which may be optionally substituted.

The term "heteroarylthio" refers to the group -S-heteroaryl.

The term "acyloxy" refers to the ester group –OC(O)-R, where R is H, alkyl, alkenyl, alkynyl, aryl, or arylalkyl, wherein the alkyl, alkenyl, alkynyl and arylalkyl groups may be optionally substituted.

The term "carboxy esters" refers to –C(O)OR where R is alkyl, aryl or arylalkyl, wherein the alkyl, aryl and arylalkyl groups may be optionally substituted.

oxygen, phosphorus and sulfur or a 5- or 6-membered ring containing from one to three heteroatoms selected from the group consisting of nitrogen, oxygen, phosphorus and sulfur; wherein the 5-membered ring has 0-2 double bounds and the 6-membered ring has 0-3 double bounds; wherein the nitrogen and sulfur atom maybe optionally oxidized; wherein the nitrogen heteroatoms may be optionally quaternized; and including any bicyclic group in which any of the above heterocyclic rings is fused to a benzene ring or another 5- or 6-membered heterocyclic ring independently defined above. Heterocyclics can be unsubstituted or monosubstituted or disubstituted with substituents independently selected from hydroxy, halo, oxo (C=O), alkylimino (R-N= wherein R is a alkyl group), amino, alkylamino, dialkylamino, acylaminoalkyl, alkoxy, thioalkoxy, polyalkoxy, alkyl, cycloalkyl or haloalkyl. Examples of heterocyclics include: imidazolyl, pyridyl, piperazinyl, azetidinyl, thiazolyl and triazolyl.

The term "glycosyl" as used herein refers to any pyranose or furanose saccharide group, including but not limited to D- or L-glucosyl, galactosyl, mannosyl, fucosyl, N-acetylneuraminyl, glucosaminyl, galactosaminyl, etc.

The term "disaccharide" as used herein refers to any pyranose or furanose saccharide group, including but not limited to D- or L-glucosyl, galactosyl, mannosyl, fucosyl, N-acetylneuraminyl, glucosaminyl, galactosaminyl, etc. linked through a glycosidic bond to another pyranose or furanose saccharide.

The term "oligosaccharide" as used herein refers to any pyranose or furanose groups including but not limited to D- or L-glucosyl, galactosyl, mannosyl, fucosyl, N-acetylneuraminyl, glucosaminyl, galactosaminyl, etc. linked through glycosidic bonds to another pyranose or furanose saccharides in which the number of saccharide groups is no less than three.

The term "glycosyl donor" as used herein refers to any pyranose or furanose saccharide or disaccharide group capable of glycosylating an acceptor such as hydroxyl, donors and includes but is not limited to suitably protected D- or L-thiotoluyl glucopyranoside, thiotoluyl galactopyranoside, mannopyranoside, fucopyranoside, N-acetylneuraminopyranoside, glucosaminopyranoside, galactosaminopyranoside, etc. The glycosidic linkages can be alpha, beta or alpha/beta mixtures. Figures 1 through 4 are examples of such saccharide and disaccharide groups.

The term "carbohydrate-activating group" as used herein refers to classes of functional groups that when attached to carbohydrates convert then into glycosyl

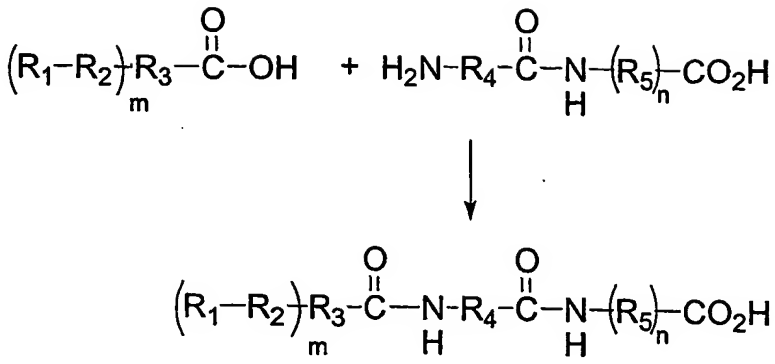
wherein said structures can replace acidic, basic, and neutral amino acids. Specifically, the introduced sugar motif can replace acidic amino acid residues if the sugar motif contains an acidic functional group, replace basic amino acid residues if the sugar motif contains a basic functional group, or replace neutral amino acids if the sugar motif contains a neutral functional group.

The term "amino-containing saccharide group" refers to a saccharide group having at least one amino substituent. Representative amino-containing saccharides include mycaminose, desosamine, L-vancosamine, 3-desmethyl-vancosamine, 3-epi-vancosamine, 4-epi-vancosamine, acosamine, actinosamine, daunosamine, 3-epi-daunosamine, ristosamine, N-methyl-D-glucamine and the like.

"Optionally substituted" groups may be substituted or unsubstituted. The substituents of an "optionally substituted" group may include, without limitation, one or more substituents independently selected from the following groups or designated subsets thereof: alkyl, alkenyl, alkynyl, heteroalkyl, haloalkyl, haloalkenyl, haloalkynyl, cycloalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, alkoxy, aryloxy, haloalkoxy, amino, alkylamino, dialkylamino, alkylthio, arylthio, heteroarylthio, oxo, carboxyesters, carboxamido, acyloxy, H, F, Cl, Br, I, CN, NO_2, NH_2, N_3, $NHCH_3$, $N(CH_3)_2$, SH, SCH_3, OH, OCH_3, OCF_3, CH_3, CF_3, $C(O)CH_3$, CO_2CH_3, CO_2H, $C(O)NH_2$, pyridinyl, thiophene, furanyl, indole, indazol, esters, amides, phosphonates, phosphates, phosphoramides, sulfonates, sulfates, sulfonamides, carbamates, ureas, thioureas, thioamides, thioalkyls. An optionally substituted group may be unsubstituted (*e.g.*, $-CH_2CH_3$), fully substituted (*e.g.*, $-CF_2CF_3$), monosubstituted (*e.g.*, $-CH_2CH_2F$) or substituted at a level anywhere in-between fully substituted and monosubstituted (*e.g.*, $-CH_2CF_3$).

The term "halogen" includes F, Cl, Br and I.

The term "protected amino", "amine protecting group" and "protected aminomethyl" as used herein refers to known amine protecting groups used in the synthetic organic chemistry art and include but are not limited to *t*-butoxycarbonyl (BOC), benzyloxycarbonyl (Cbz), azide (N_3), 2-trimethylsilylethoxycarbonyl (Teoc), allyloxycarbonyl (Alloc), 9-fluorenylmethyloxycarbonyl (Fmoc), acyl groups, such as formyl, acetyl, trihaloacetyl, benzoyl, and nitrophenylacetyl, sulfonamide groups, imine- and cyclic imide groups. Further examples of protected amino groups are



where R_1 is any carbohydrate including mono-, di-, tri-, and tetrasaccharides and larger, which may contain one or more amino sugars, deoxy sugars or sialic acid sugars in any combination and in which any hydroxyl, amino or carboxyl functions are suitably modified by sulfation, alkylation, acylation, deoxygenation, diazotization, pegylation, silylation and the like;

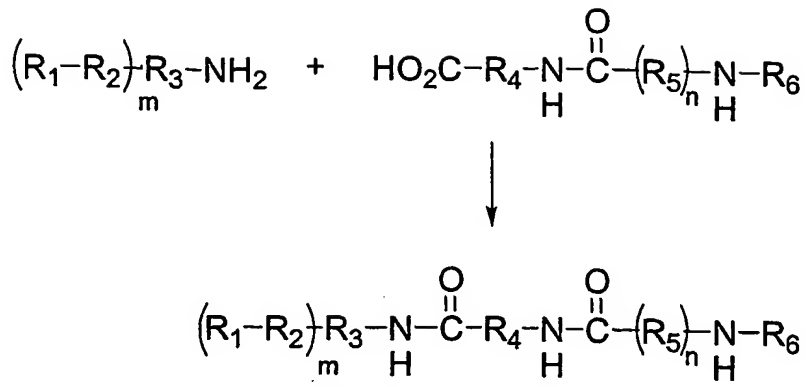
R_2 is the atom or group at the anomeric position of the carbohydrate R_1 and may be O, S, NH or CH_2;

R_3 is a linker composed of alone or in any combination alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, alkoxy, aryloxy, alkylthio, arylthio, aryl, heteroaryl, heteroarylalkyl, heteroarylthio, acyloxy, carboxyesters, carboxamido, arylalkyl, haloalkyl, haloalkenyl, haloalkynyl, haloalkoxy, cycloalkyl, acyl, alkylacylamino or acylamino groups or amino acid residues;

R_4 and R_5, when substituted with NH_2 and CO_2H, are any natural amino acid or amino acid surrogate in which any reactive groups are suitably protected;

m is 1, 2, or 3; and n is any integer from 1 to about 100, but may be greater.

In another preferred embodiment of the present invention, one or more protected carbohydrates are conjugated through a linker to hydroxyl or amine functions on the side chains of amino acids along the backbone of a suitably protected peptide *via* a dehydration reaction thus:



where R_1 is any carbohydrate including mono-, di-, tri-, and tetrasaccharides and larger, which may contain one or more amino sugars, deoxy sugars or sialic acid sugars in any combination and in which any hydroxyl, amino or carboxyl functions are suitably modified by sulfation, alkylation, acylation, deoxygenation, diazotization, pegylation, silylation and the like;

R_2 is the atom or group at the anomeric position of the carbohydrate R_1 and may be O, S, NH or CH_2;

R_3 is a linker composed of alone or in any combination alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, alkoxy, aryloxy, alkylthio, arylthio, aryl, heteroaryl, heteroarylalkyl, heteroarylthio, acyloxy, carboxyesters, carboxamido, arylalkyl, haloalkyl, haloalkenyl, haloalkynyl, haloalkoxy, cycloalkyl, acyl, alkylacylamino or acylamino groups or amino acid residues;

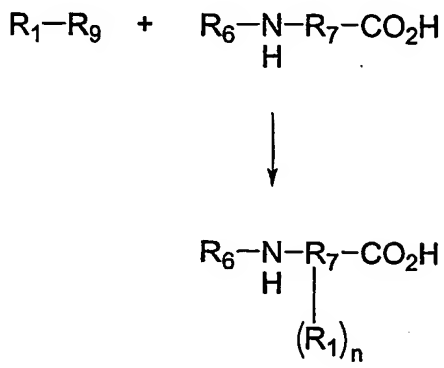
R_4 and R_5, when substituted with NH_2 and CO_2H, are any natural amino acid or amino acid surrogate in which any reactive groups are suitably protected;

R_6 is a protecting group for an amine including but not limited to Fmoc, Boc, Cbz and the like;

m is 1, 2, or 3; and n is any integer from 1 to about 100, but may be greater.

In another preferred embodiment of the present invention, one or more protected carbohydrates are conjugated through a linker to carboxyl functions on the side chains of amino acids along the backbone of a suitably protected peptide *via* a dehydration reaction thus:

In another preferred embodiment of the present invention, a protected carbohydrate is conjugated to a hydroxy or amino group on the side chain of an amino acid along the backbone of a suitably protected peptide *via* direct glycosylation thus:



where R_1 is any carbohydrate including mono-, di-, tri-, and tetrasaccharides and larger, which may contain one or more amino sugars, deoxy sugars or sialic acid sugars in any combination and in which any hydroxyl, amino or carboxyl functions are suitably modified by sulfation, alkylation, acylation, deoxygenation, diazotization, pegylation, silylation and the like;

R_6 is a protecting group for an amine including but not limited to Fmoc, Boc, Cbz and the like;

R_7, when substituted with NH_2 and CO_2H, is any suitably protected natural or synthetic peptide containing one or more amino acid residues with side chains bearing a hydroxyl or amine function such as serine, threonine, hydroxyproline, tyrosine, lysine, hydroxylysine, arginine, or any other amino acid surrogates containing a hydroxyl or amine function on the side chain;

R_9 is a sugar activating group such as but not limited to sulfide, trichloroacetimidate, bromide, chloride, fluoride, acyloxy, sulfoxide, phosphite and the like;

and n is any integer from 1 to about 100, but may be greater.

Thus, with respect to the above described preferred embodiments, the present invention encompasses both the method of making such glycoconjugates as well as the resulting glycoconjugate compounds and compositions.

acid or any other amino acid surrogate bearing a hydroxyl, amino, or carboxyl group on the side chain.

The reaction between the carboxylic acid or amine group on the carbohydrate linker and the peptide can be promoted with the use of a coupling agent such as, 2-(1*H*-9-azobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU); 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU); 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU); benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP); benzotriazole-1-yl-oxy-trispyrrolidinophosphonium hexafluorophosphate (PyBOP); 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI); *N-N'*-dicyclohexylcarbodiimide (DCC) and the like.

Alternatively the peptide may be directly glycosylated with one or more carbohydrate moieties at the side chain hydroxyl or amino group of an amino acid, which may be serine, threonine, hydroxyproline, tyrosine, lysine, proline or arginine or any other amino acid surrogate bearing a hydroxyl or amino group on the side chain. Glycosidic bond formation may be achieved through the use of OPopS™ technology (WO000/09527) in a one-pot fashion in which the final acceptor added to the reaction is a peptide containing a free hydroxyl or amino group.

Global deprotection of the glycoconjugate using reagents and conditions well known to one skilled in the art affords a new class of biologically active peptides whose half-lives may be determined through assays with animal tissue homogenate or plasma or commercially available enzymes known to degrade peptides such as but not limited to trypsin, chymotrypsin, alanyl aminopeptidase, lysine aminopeptidase, leucine aminopeptidase and prolyl carboxypeptidase and the like. The half-lives may be measured using a variety of analytical techniques such as mass spectrometry, gas chromatography, high performance liquid chromatography, gas chromatography/mass spectrometry or liquid chromatography/mass spectrometry. By comparison of the half-life of a glycopeptide with that of the native peptide from which it was derived the effect of glycoconjugation on the peptide's stability towards peptidase enzymes can be demonstrated.

The three-dimensional conformation of the glycopeptides of this present invention is substantially similar to their corresponding peptides which contain amino acids and no sugars. This three-dimensional structure can be determined by

acid. The salt is then isolated by evaporating the solution. In another example, the salt is prepared by reacting the free base and acid in an organic solvent.

Carriers or excipients can be used to facilitate administration of the compound, for example, to increase the solubility of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. In addition, the molecules tested can be used to determine the structural features that enable them to act on the *ob* gene control region, and thus to select molecules useful in this invention. Those skilled in the art will know how to design drugs from lead molecules, using techniques such as those disclosed in PCT publication WO 94/18959, incorporated by reference herein.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

For any glycopeptide used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal disruption of the protein complex, or a half-maximal inhibition of the cellular level and/or activity of a complex component). Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl et al., in

by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

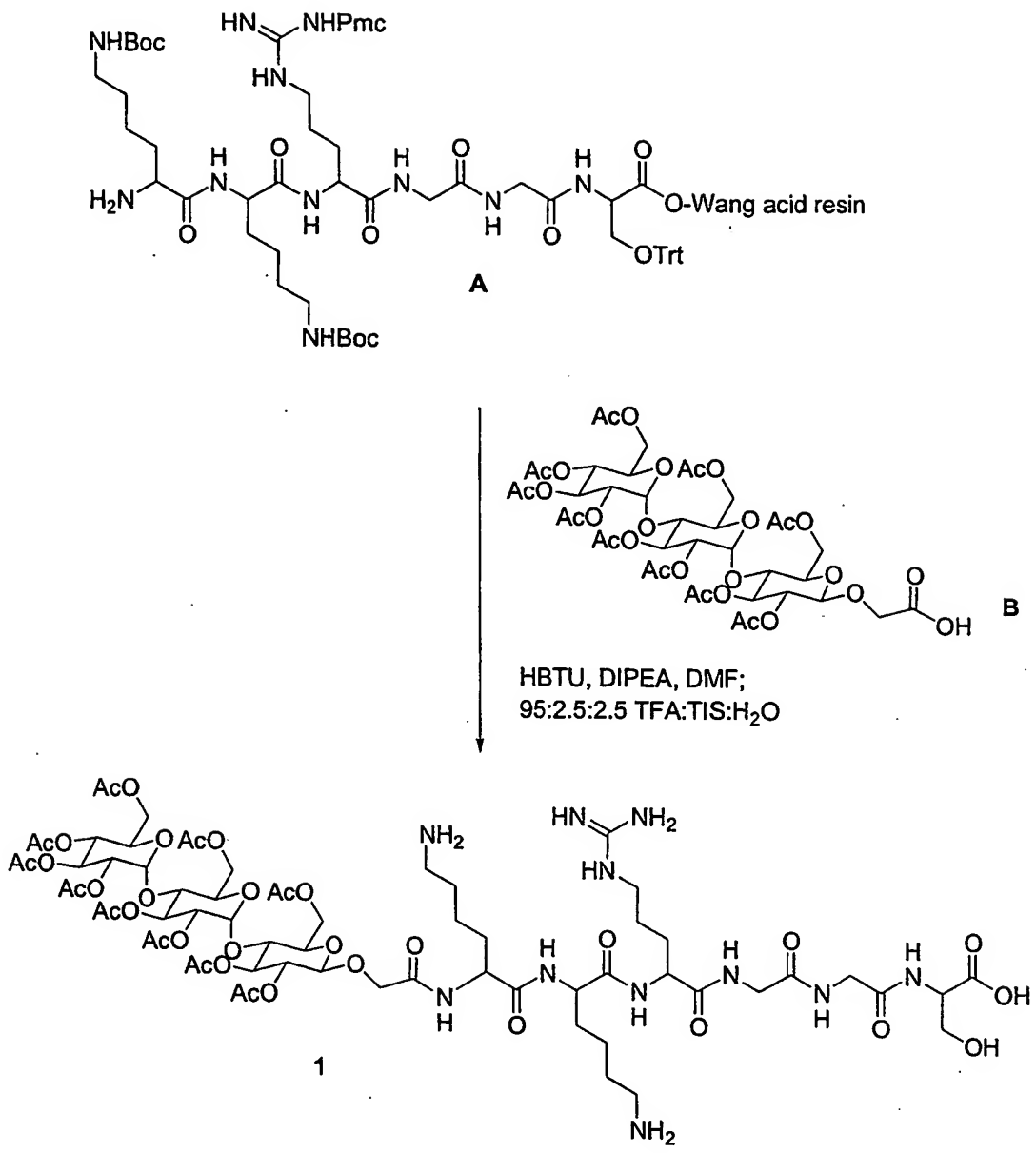
Agents intended to be administered intracellularly may be administered using techniques well known to those of skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran.

variations of the present invention. The materials, methods, and examples are illustrative only and not intended to be limiting.

Scheme 1


NHBoc
HN
NHPmc
HN
H2N
O-Wang acid resin
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NHBoc
A
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HBTU, DIPEA, DMF;
95:2.5:2.5 TFA:TIS:H2O
NH2
HN
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HN
OH
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NH2
1

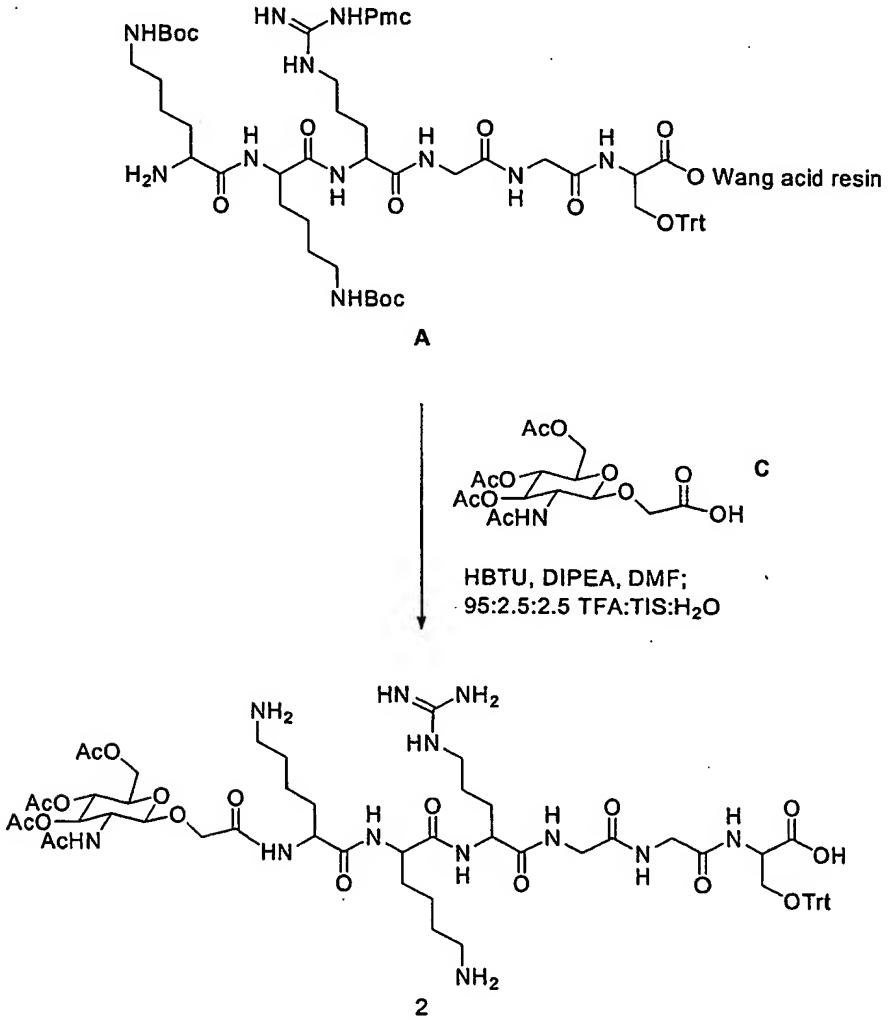
5

Example 2

Synthesis of 2: Prepared using the procedure described in Example 1 from peptide **A (SEQ ID No. 1)** and carbohydrate glycolic acid **C** to give the crude triphenylmethyl protected glycopeptide **2** (Scheme 3). MS (ES^+) *m/z* 642 $(M + H + Na)^{2+}$ ($C_{60}H_{84}N_{12}NaO_{18}$).

Degradation study: Compound **2** was degraded using trypsin as described in Example 1 (Scheme 4). The data are summarized in Table 1.

Scheme 3

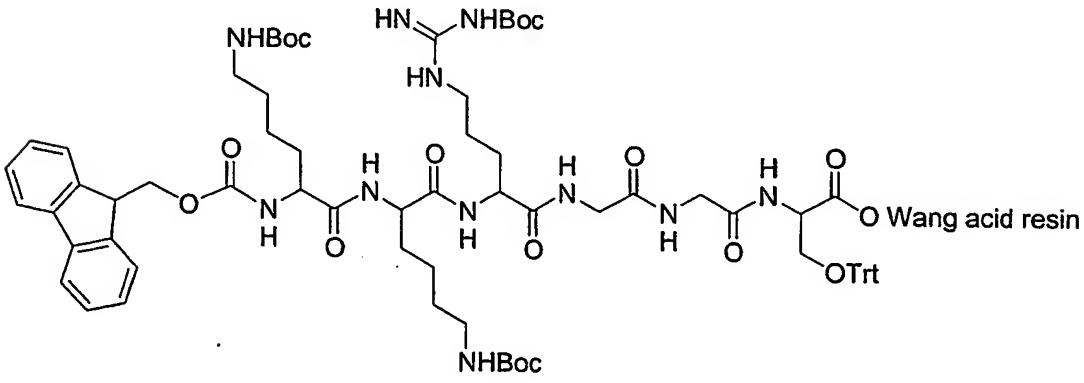


Example 4

Synthesis of 4. Peptide **D** (Protected Peptide **A: SEQ ID No. 1**)(200 mg) was treated with 95:2.5:2.5 TFA:TIS:H_2O for 90 minutes. The mixture was filtered and the filtrate was concentrated under reduced pressure to leave the crude flourenylmethyloxycarbonyl (Fmoc) protected peptide **4** (Scheme 6). MS (ES^+) *m/z* 854 $(M + H)^+$ ($C_{40}H_{59}N_{11}O_{10}$).

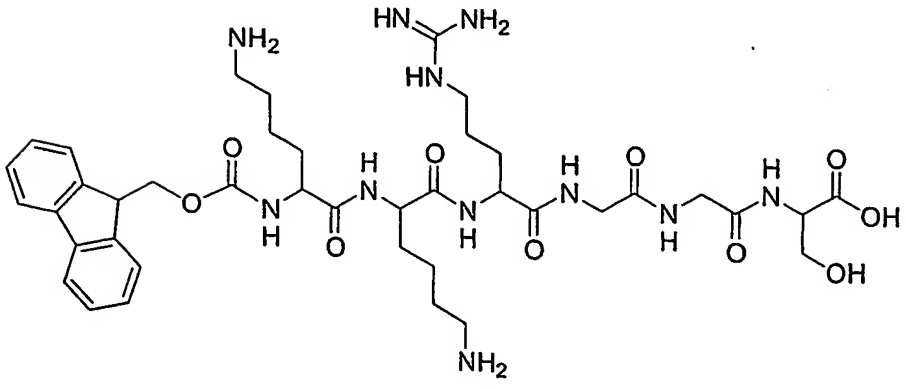
Degradation Study: Compound **4** was degraded using trypsin as described in Example 1 (Scheme 7). The data are summarized in Table 1.

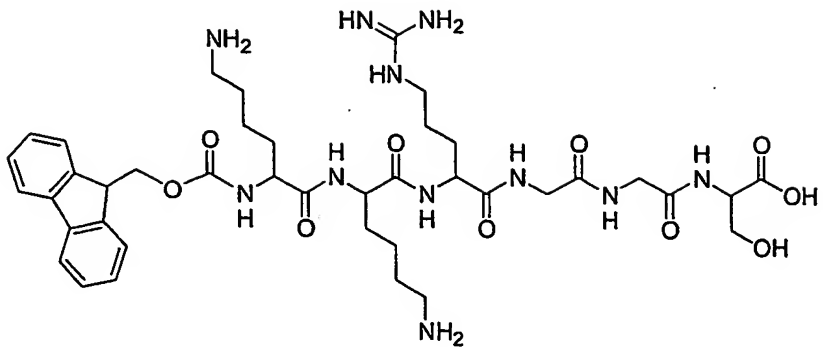
Scheme 6



D

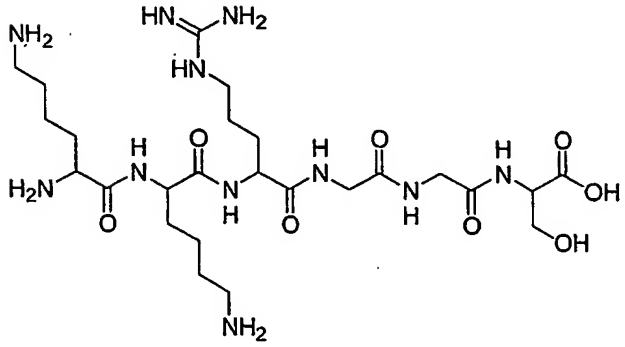
95:2.5:2.5 TFA:TIS:H_2O



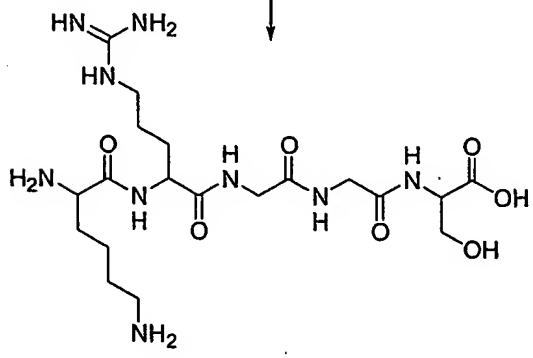


4

Parent



Deg 1



Deg 2